

The 3'-5' ero plus F $\rightarrow$ Y Mut. Project N \_\_\_\_\_  
Book No. \_\_\_\_\_

Pag N. \_\_\_\_\_

cline

Replace mutant fragment from RF to pUC19-Tne.

I can not do it directly because there are two Sph I sites in pTNE.

Therefore, first replace the mutant fragment in pUCTNE and then transfer into pTNE.

Digest pUCTNE with SphI + HindIII. Digest M13mp19 mutants with SphI + HindIII.

+ 2 3 4

1 = RF 3'-5' ero - SphI / HindIII

2 = pTNE

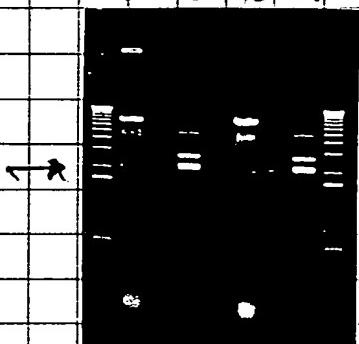
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3 = RF F $\rightarrow$ Y mutant

"

4 = pTNE

"



Purify only mutant fragment - to be ligated into pUCTNE SphI / HindIII frag.

5 & 6

5 & 6 pUCTNE SphI / HindIII



Purify the largest fragment. Ligate with the mutant fragments:

Transform DH5B.

Plate 10% & 90%

Overshoot at 30°

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ssed & Understood by me,

Date

Invented by

Date

H. H. J.

6/12/95

Recorded by

Debra Rutherford

5/1/95